Amplifluor™ Universal Detection System

The Amplifluor™ Universal Detection System is a proprietary technology platform that allows the simultaneous amplification and detection of nucleic acids within a closed reaction vessel. The method is based upon the incorporation of energy transfer-labeled hairpin primers into the amplification product. Amplifluor™ hairpin primers are designed so that a fluorescent signal is generated only when the primer is unfolded during its incorporation into an amplification product. The fluorescence signal produced directly correlates with the accumulation of PCR product at each cycle. Unincorporated Amplifluor™ primers have an extremely low fluorescence signal eliminating the need to purify the PCR reaction prior to quantitation; therefore, PCR and fluorescent signal detection can occur in a single reaction vessel. Signal is measured either during the reaction (real-time) or after the last cycle of the reaction (endpoint). Amplifluor™ primers also perform extremely well for *in situ* PCR applications using paraffin-embedded tissues (see References utilizing Amplifluor™ Technology).

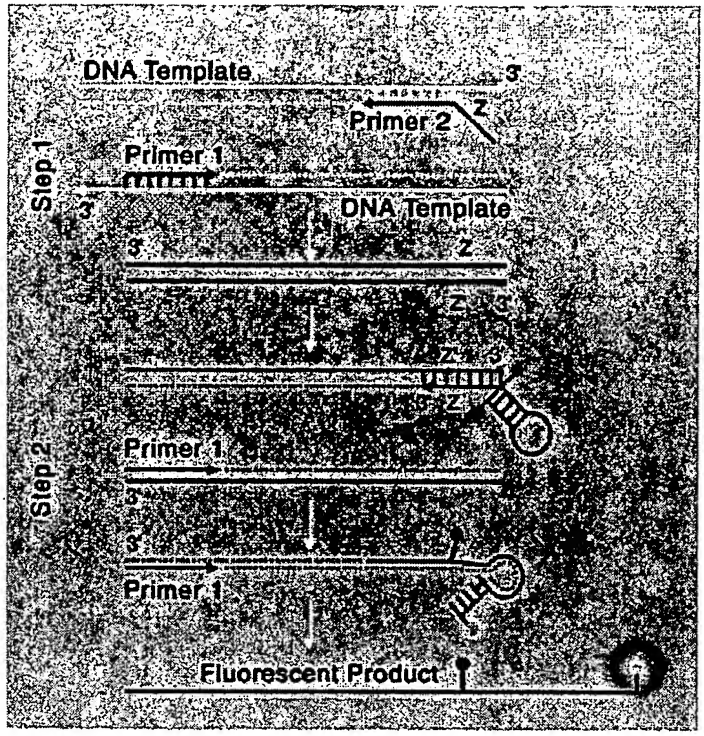
Features

- Fluorescence signal correlates directly with the accumulation of PCR product
- Direct fluorescence detection simplifies data interpretation
- High sensitivity and broad dynamic range
- Specific, one-step reaction eliminates the need for hybridization probes
- Cost-effective quantitative PCR system
- Universal primer applicable to all DNA and mRNA targets in solution-phase or *in situ*
- Closed-tube assay eliminates the chance of PCR contamination
- Can be used in most real-time or endpoint fluorescence detection instruments
- Multiplex color options available through custom synthesis
- Universal kit includes positive control template and primers for assay validation

Principles of the Amplifluor™ Universal Detection System Procedure

The Amplifluor™ Universal Detection System is based on molecular energy transfer from an excited fluorophore to an acceptor moiety that results in quenching of the fluorescence emission. In the Amplifluor™ Universal System, quenching is facilitated by tethering the fluorophore (fluorescein) and the acceptor 4-(4'-dimethylamino-phenylazo) sulfonic acid (DABSYL) together via an oligonucleotide primer. This oligonucleotide primer is called the UniPrimer™ Energy Transfer-labeled Primer because it provides the user with a universal format in which to detect PCR products using fluorescence energy transfer.

UniPrimer™ Energy Transfer-labeled Primer: the unique design of UniPrimer™ is such that it emits a fluorescence signal only upon incorporation into the amplification products produced during each PCR cycle. Low levels of amplification products can be detected and quantified directly on endpoint or real-time fluorescence instruments since the unincorporated UniPrimer™ does not fluoresce.



UniPrimer™ consists of a 3' 18 base oligonucleotide tail (Z sequence) and a 5' intracomplementary sequence labeled with a pair of energy transfer molecules. The "Z sequence" acts as a universal PCR primer and is specifically designed to reduce PCR background due to heterodimer formation. To use the UniPrimer™ in PCR, the Z sequence is added to the 5' end of one of the target-specific primers. As shown in the figure, the UniPrimer™ anneals to the complement to the Z sequence (Z' sequence) contained in an amplicon generated in the initial cycles of the reaction. As the UniPrimer™ is incorporated and the hairpin is unfolded, quenching is no longer possible due to the increased distance between the fluorescein and DABSYL moieties. The fluorescence signal produced with each PCR cycle directly correlates to the amount of amplified DNA generated allowing for quantitation over a wide target range.

References utilizing Amplifluor™ Technology

1. Detection of telomerase activity utilizing energy transfer primers: Comparison with gel-and ELISA-based Detection. Uehara, H. et al. 1999. *Biotechniques* 26(3), 552-558
2. In Situ Amplification Using Universal Energy Transfer-labeled Primers. Nuovo, G.J. et al. 1999. *The Journal of Histochemistry and Cytochemistry* 47(3), 273-279.
3. Direct fluorescence detection of allele-specific PCR products using novel energy-transfer labeled primers. Winn-Deen, E.S. 1998. *Molecular Diagnosis* 3(4), 217-222.
4. A closed tube format for amplification and detection of DNA based on energy transfer. Nazarenko, I.A. et al. 1997. *Nucleic Acids Research* 25(12), 2516-2521.

Protocols

- Assay setup for endpoint or real-time protocols.
- Setting up real-time instruments.

Technical Notes

- Troubleshooting

- Helpful hints for primer design.

Instrument Options

Amplifluor™ technology is uniquely compatible with virtually any instrument that measures fluorescence.

Real-time Detection Instruments:

Real-time detection instruments measure fluorescence accumulation at each cycle of PCR. When using a real-time measuring device with UniPrimer™ reactions, program the instrument to measure fluorescence during the annealing step at temperatures between 50-60°C. At this temperature, unincorporated UniPrimer™ is in the silent hairpin conformation and will not contribute to fluorescence measurements. Amplifluor™ technology has been used successfully on the ABI PRISM™ 7700, the LightCycler™, and the iCycler™ IQ.

Endpoint Detection Instruments:

Fluorescence Plate Reader:
In this option, PCR is performed using any temperature cycler then read directly on a fluorescence plate reader without having to transfer the samples from the PCR vessel to another plate format, such as a microtiter plate. Amplifluor™ Systems are optimized for maximum sensitivity in the fluorescence plate reader format. The instruction manuals specify cycling and reaction conditions for single point readings taken at the end of the reaction (endpoint). These single point determinations are made in the exponential amplification range of PCR. Advantages of this method are:

- Closed-tube method of amplification and detection
- Direct sample readout facilitates quantitation
- Advanced plate design facilitates higher throughput than many real-time instruments

Spectrofluorometer:
The yield of the PCR reaction may be determined by placing a diluted aliquot of each completed reaction in a cuvette and measuring the fluorescence in a spectrofluorometer. To read fluorescence, set the excitation setting to 495 nm and the emission setting to 516 nm.

UV transilluminator/camera:
Amplifluor™ reactions are easily visualized using this method. PCR is performed in standard reaction vessels (tubes, tube strips or plates) or in the optional gel format. Because the amplicon is directly labeled with fluorescence, no ethidium bromide is required for visualization. (Please inquire regarding the use of filters with your standard camera or CCD-based gel imaging system). Though not as quantitative as other instrument options, this method provides a rapid, "yes" or "no" answer for researchers wanting to confirm the success of their amplification reactions. Additionally, this option is useful when constructing gene-specific primers required for the Amplifluor™ Universal Detection System (#S7901).

Fluor imaging gel systems:
This method is used similarly to the method above but is a more quantitative method. We have used the FMBIO® II successfully for several Amplifluor™ gel-based analysis assays.

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Amplifluor™ Universal Detection System Components

The Amplifluor™ Universal Detection System (# S7901) provides enough reagents to perform 100 PCR reactions with any gene target by simply adding a unique oligonucleotide sequence (designated Z) to one of the target-specific primers. Target-specific control template and primers for *bcl-2* are supplied (20 reactions) to aid in qualification of the PCR reagents that are not included with the kit (such as polymerase, buffer and dNTPs) and the fluorescence measuring device. (Reaction sizes of 1,000 and 10,000 are also available).

Components

1. 10X UniPrimer™ Energy Transfer-labeled Primer, fluorescein (5.0 μM) (light sensitive)

2. Control Template and Primers
   - 10X Tailed Primer (0.5 μM)
   - 10X Untailed Primer (5.0 μM)
   - Control Template,1 ng/μl (supplied at 2 x 10^8 copies/μl)

Kit Components
Ordering Information

Return to Home Page